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Prevalence of *Arcobacter* species in market-weight commercial turkeys

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Abstract The prevalence of *Arcobacter* in live market weight turkeys was determined for six Midwestern commercial flocks at three intervals. Samples (n = 987) were collected from cloaca, feathers, ceca, crop, drinkers and environmental samples on farms and from carcasses at slaughter. Initially, EMJH-P80 and CVA isolated *Arcobacter* from 7.1% (40 of 564) of samples, while *Arcobacter* enrichment broth and selective agar recovered the microbe in 4.7% of samples (23 of

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Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA 489 samples). Although EMJH-P80 coupled with CVA yielded Arcobacter more frequently, the selectivity of the modified Arcobacter agar enhanced the recognition of Arcobacter colonies. A multiplex PCR was used to identify all Arcobacter species and to differentiate Arcobacter butzleri. The low prevalence of Arcobacter detected in cloacal swab (2.0%, 6 of 298 samples) and cecal contents (2.1%, 3 of 145 samples) suggests that Arcobacter infrequently colonizes the intestinal tract. Despite its low prevalence in live turkeys, Arcobacter spp. were identified in 93% of carcass swabs (139 of 150 samples). The overall prevalence of Arcobacter in drinker water decreased from 67% (31 of 46 samples) in the summer of 2003 to 24.7% (18 of 73 samples) during resampling in the spring of 2004 and was inversely related to the chlorination level.

Keywords Arcobacter spp. · Ceca · Environmental sampling · Turkeys · Water

Introduction

Arcobacter spp. were once classified as aerotolerant campylobacteria (Neill et al. 1985), but were proposed as a new genus based on their ability to grow in air (aerotolerance) at 25°C (Vandamme et al. in 1991). Of the seven recognized species, A. butzleri, A. cryaerophilus,



A. skirrowii, and recently A. cibarius have been reported in poultry, livestock, carcasses or their meat products (Ho et al. 2006; Houf et al. 2005; Kabeya et al. 2004; Lehner et al. 2005; Ongor et al. 2004; Wesley et al. 2000). A. buztleri and rarely A. cryaerophilus, A. skirrowii have been recovered from cases of human gastroenteritis, diarrhea (Kiehlbauch et al. 1991a; Lehner et al. 2005; Taylor et al. 1991; Vandamme et al. 1992; Wybo et al. 2004), and bacteremia (Hsueh et al. 1997; Vandenberg et al. 2004; Woo et al. 2001). Surveillance of human clinical stool samples in France and Belgium ranked A. butzleri as the fourth most frequently isolated Campylobacteraceae (Prouzet-Mauleon et al. 2006; Vandenberg et al. 2004).

A. butzleri and A. cryaerophilus are found on poultry carcasses (Atabay et al. 1998; Houf et al. 2002; Scullion et al. 2006), suggesting that consumption of contaminated products is a risk factor for human infection. The serogroup identity of A. butzleri isolates from poultry and clinical cases of diarrhoeal illness suggested that poultry are possible reservoir of infection (Marinescu et al. 1996). Poultry, including up to 97% of chicken carcasses (Atabay et al. 2003; DeBoer et al. 1996; Kabeya et al. 2003; Lammerding et al. 1996), retail products (Scullion et al. 2006), and up to 80% of ground poultry (Manke et al. 1998; Rivas et al. 2004; Scullion et al. 2004) are often contaminated with Arcobacter spp., primarily A. butzleri. In one study, A. cryaerophilus was the only Arcobacter species recovered from poultry transport crates (Houf et al. 2002).

Despite the frequency of recovering *Arcobacter* from carcasses and the slaughterhouse environment (Gude et al. 2005; Houf et al. 2002; Son et al. 2006) isolations from live birds are infrequent. This suggests that colonization may be transient since following experimental inoculation *Arcobacter* spp. were recovered from 75–100% of environmental drag samples, but not from the gastrointestinal tract of bird (Eifert et al. 2003). In another study involving experimentally inoculated young birds, *Arcobacter* spp. were recovered from 65% of the highly inbred Beltsville White turkey poults but less frequently from either conventional turkey poults (6%) or conventional broiler chicks (0%, Wesley and Baetz 1999).

Although A. butzleri was absent from chickens and their house environment, it was isolated from effluent and stagnant water outside the poultry houses, the slaughterhouse environment and on chicken carcasses (Gude et al 2005). Sporadic colonization of Arcobacter in the live bird (Atabay and Corry 1997; Atabay et al. 1998; Corry and Atabay 2001; Eifert et al 2003; Houf et al. 2002) contrasts with the frequency of Campylobacter jejuni and Campylobacter coli in the avian intestine. Arcobacter spp. survives in the environment of the broiler slaughterhouse, including processing water, despite the plant's cleaning and descaling schedule (Houf et al. 2003). Daily variations of PFGE profiles infer that in-coming birds, in addition, may introduce new strains into the abattoir (Son et al. 2006). Thus, Arcobacter spp. may potentially survive in the environment to cross-contaminate in-coming birds.

Arcobacter have been cultured from a variety of water sources, including marine, estuary and brackish environments reflecting its ability to survive in these hostile domains (Dhamabutra et al. 1992; Diergaardt et al. 2004; Donachie et al. 2005; Fera et al. 2004; Ho et al. 2006; Jacob et al. 1993; Maugeri et al. 2004, 2005; Morita et al. 2004; Musmanno et al. 1997; Wirsen et al. 2002). Thus, contaminated water may be a potential vehicle of transmission and a major risk factor in acquiring diarrhoeal illness (Andersen et al. 1993; Festy et al. 1993; Kiehlbauch et al. 1991b; Lerner et al. 1994; Rivas et al. 2004). Although it remains viable in non-chlorinated drinking water for up to 16 days, chlorination inactivated Arcobacter within 5 min (Moreno et al. 2004). Following its isolation in well water supplying a youth summer camp experiencing an outbreak of gastroenteritis, Rice et al. (1999) advised that continuous chlorination was the only effective barrier to the spread of A. butlzeri from contaminated water sources.

Previous reports have described protocols to estimate *Arcobacter* prevalence in live birds or poultry meat (DeBoer et al. 1996; Eifert et al. 2003; Houf et al. 2001; Johnson et al. 1991; Lerner et al. 1994; Phillips 2001; Ridsdale et al. 1998; Scullion et al. 2004; Son et al. 2006). Because of its complexity, few studies have evaluated Ellinghausen-McCollough-Johnson-Harris Polysorbate 80



(EMJH-P80) supplemented with 5-fluorouracil (Johnson and Murano 1999; Son et al. 2006). EMJH-P80 was used in the initial description of aerotolerant campylobacteria in aborted livestock fetuses (Ellis et al. 1977). Houf et al. (2001) reported that the selective agents (5-fluorouracil, amphotericin B, cefoperazone, novobiocin and trimethoprim) suppressed growth of background flora, facilitating the identification of Arcobacter. The formulation of Johnson and Murano (1999), which utilized charcoal, 5-fluorouracil, bile salts, and cefoperazone, improved recovery of Arcobacter when compared to EMJH-P80 and brain heart infusion (BHI) agar containing ceforperazone, vancomycin, amphotericin, and blood (CVA). In this study, we wished to evaluate EMJH-P80 followed by plating to CVA with the selective enrichment and agar of Houf (2001).

Therefore, the goals of this study were (i) to optimize the *Arcobacter* detection protocol for use in live turkeys, and (ii) to estimate the prevalence of *Arcobacter* in live commercial market weight turkeys and the flock environment.

Materials and methods

Study farms

Six commercial turkey farms in the Midwest were chosen for sampling in the summer of 2003 and 2004. Poults were obtained from four different commercial hatcheries. All the farms utilized a two-stage growing program except Farm 5, which used a three-stage growing program. Feed was purchased from four different feed mills, and every farm incorporated growth-promoting antibiotics as feed additives. Litter consisted of oat hulls, wood shavings, or a combination of the two. There was wide variation in litter management ranging from fresh litter to two-year old litter. Water sources included both city and farm well water, and ranged in chlorination levels from constant to none. All farms reported rodents in the house. Half of the farms utilized footbaths as part of their biosecurity program while the remainder did not.

Summer 2003 prevalence study

On-farm, cloacal swabs (n = 298) were taken with two 6-inch sterile cotton-tipped applicators (Harwood Products, Gilford, Maine) inserted 7.5 cm into the cloaca. Each swab was then placed into a tube containing 9 ml of either EMJH-P80 or *Arcobacter* enrichment broth. The end of the swab was broken off to prevent contamination.

Breast feather swabs (n = 75) were obtained using sterile 1.5×30 -inch Speci-sponge (Nasco, Ft. Atkinson, Wisconsin) hydrated in 15 ml of sterile Buffered Peptone Water (Oxoid, Hampshire, England). The feathers along the sternum were wiped for approximately 15 sec and swabs placed in sterile Whirl-Pac bags (Nasco, Ft. Atkinson, WI). Sponges were processed within 12 h by aseptically cutting each sponge in half, and placing a half into 27 ml of either EMJH-P80 or *Arcobacter* enrichment broth. Scissors were flamed between sponge samples.

Drinkers (n = 46) were sampled by collecting 2 ml of water with a sterile pipette, and inoculating 1 ml into 9 ml of both EMJH-P80 and *Arcobacter* enrichment broth.

Environmental samples (n = 25) of one turkey house where obtained by swabbing walls and litter with 4×4 -inch gauze (Johnson and Johnson, New Brunswick, New Jersey) moistened with Buffered Peptone Water (Oxoid, Hampshire, England). Gloves were changed after obtaining each sample to prevent cross-contamination.

Swabs (breast feathers, environmental samples) where processed within 12 h by aseptically cutting a 1×1 -inch piece from the gauze and placing it into 9 ml of EMJH-P80. Scissors were flamed between swabs.

At slaughter, ceca (n = 70) were collected and placed in Whirl-Pac bags. At the laboratory, one gram of cecal contents was added to 9 ml of both EMJH-P80 and *Arcobacter* enrichment broth. Crops (n = 50) were removed by plant personnel and placed into Whirl-Pac bags (Nasco, Ft. Atkinson, Wisconsin) for transport to the laboratory. A suspension was prepared by adding 10 ml of sterile Buffered Peptone Water (Oxoid, Hampshire, England) to each crop and contents manually massaged for 30 sec.



Summer 2003 media comparison

For media comparisons, 1 ml of the crop suspension was placed in 9 ml of both EMJH-P80 and incubated microaerobically at 30°C for 7 days (Johnson and Murano 1999) and in modified *Arcobacter* enrichment broth at 28°C for 48 h (Houf et al. 2001). The enrichments were plated onto either CVA or *Arcobacter* selective agar (Houf et al. 2001), respectively. Both sets of plates were incubated microaerobically (5% O₂, 10% CO₂, 85% N₂) for an additional 48 h at their respective temperature. Suspect colonies were transferred onto 10% defibrinated sheep blood agar plates and incubated microaerobically (5% O₂, 10% CO₂, 85% N₂).

Presumptive *Arcobacter* colonies were screened using multiplex PCR, which targets the 23S rRNA of all *Arcobacter* species and the 16S rRNA of *A. butzleri* (Harmon and Wesley 1997). Isolates, which were confirmed as *Arcobacter* but did not amplify with the *A. butzleri*-specific primers, were designated *Arcobacter* spp.

Spring 2004 prevalence study

Cecal (n = 75), crop (n = 75) and water samples (n = 73) were collected in the same manner as outlined in the media comparison study. A modified EMJH-P80 protocol was used to detect *Arcobacter* spp. as follows. After enrichment $(30^{\circ}\text{C for 7 days})$, a 1 ml aliquot of EMJH-P80 was centrifuged (Sorvall SA300 rotor, 11,000 rpm for 3 min.), the pellet resuspended in 50 µl of sterile distilled water, heated $(100^{\circ}\text{C for 10 min})$ and the mixture re-centrifuged (Sorvall SA300 rotor, 11,000 rpm for 3 min). An aliquot from the resultant supernatant served as a template (5 µl) for the multiplex PCR reaction (Harmon and Wesley 1997).

Summer 2004 prevalence study

Environmental samples (n = 50) from two study farms, including walls, fans, drinkers and litter, were collected as described in the Spring 2004 prevalence study. In addition, the ventral and dorsal surfaces of the carcasses (n = 150) originating from these premises were swabbed with a

sterile Speci-sponge (Nasco, Ft. Atkinson, Wisconsin) after evisceration and prior to reaching the chiller. Sponges were enriched in EMJH-P80 and screened using the protocol described in the Spring 2004 study.

Results and discussion

The recovery of Arcobacter by two isolation protocols is summarized in Table 1. For sampling completed in summer 2003, EMJH-P80 recovered Arcobacter from 7.1% (40 out of 564) of samples whereas Arcobacter enrichment broth and selective agar of Houf et al. (2001) recovered Arcobacter from 4.7% (23 of 489) of these same samples. Despite the higher isolation rates of Arcobacter from EMJH-P80, Arcobacter enrichment broth and selective agar inhibited the background competing flora and thus facilitated the recognition of Arcobacter. The majority of the colonies that grew on the selective agar were identified as Arcobacter whereas enrichment in EMJH P-80 followed by plating to CVA resulted in more background contaminants.

The multiplex PCR, which we used in this study, amplifies the 23S rRNA sequence of *Arcobacter* spp. and the 16S rRNA fragment of *A. butzleri* (Harmon and Wesley 1997). *Arcobacter* isolates, which were not *A. butzleri* were designated *Arcobacter* spp. Although we assume that these are most likely *A. cryaerophilus* based on its frequency in livestock (for example, Houf et al. 2002; Kabeya et al. 2003; Scullion et al. 2006; Son et al. 2007; VanDreiessche et al. 2003, 2004, 2005), this could not be verified with the multiplex PCR employed in this study.

As shown in Table 1, *A. butzleri* was detected in 2.0% of cloacal swabs (6 of 298 samples), 2.9% of ceca (2 of 70 samples), and 2.7% of feather swabs (2 of 75 samples). For these same flocks at the same sampling interval, *Campylobacter* spp. were recovered from 65 to 80% of cloacal swabs (n = 600), 86% of crops (n = 84) and 100% of ceca (n = 96) (Wesley et al. 2005).

In contrast to its low recovery from turkeys (Table 1), *Arcobacter* spp. were identified in 63% of water drinker samples (29 of 46 samples). The overall prevalence of *Arcobacter* spp. in water



Table 1 Summary of the
distribution of
Arcobacter: Summer 2003

Sample type	Number	Number positive samples (% positive)		Total
		ЕМЈН-Р80	Arcobacter selective media	
Cloaca swab	298	4 (1.3%)	2 (0.7%)	6 (2.0%) ^a
Cecal contents	70	1 (1.4%)	1 (1.4%)	$2(2.9\%)^{a}$
Crop	50	0 (0%)	ND^b	0 (0%)
Feathers	75	2 (2.7%)	1 (1.3%)	$(2.7\%)^{a}$
Water	46	29 (63.0%)	19 (41.3%)	29 (63.0%)
Environment	25	4 (16%)	ND	$4(16\%)^{a}$
Total	564	40 (7.1%)	23 (4.7%)	43 (7.7%)

Data are shown as a percentage of positive samples

a Isolates identified as

A. butzleri

Table 2 Prevalence of *Arcobacter* in water supply: Summer 2003

Farm number	Number positive water samples (% positive)		Water source	Chlorination status
	A. butzleri	Arcobacter spp.1		
Farm #3	6/6 (100%)	0/6 (0%)	Well	No
Farm #4	20/20 (100%)	2/20 (10%)	Well	No
Farm #5	5/10 (50%)	0/10 (0%)	Well	Not that day
Farm #6	0/10 (0%)	0/10 (0%)	City	Yes
Total	31/46 (67%)	15/46 (33%)	,	

Data are shown as a percentage of positive samples

declined from summer 2003 (67%, 31 of 46 samples, Table 2) to spring 2004 (25%, 18 of 73 samples, Table 3) with no evident change in farm management practices. Of the farm practices surveyed, the frequency of chlorination appeared to be directly related to the presence

Table 3 Distribution of *Arcobacter* in water, crop and ceca: Spring 2004

Sample						
Type Number		Number po positive)	Total			
		A. butzleri	Arcobacter spp. ¹	_		
Water	73	0 (0%)	18 (24.7%)	18 (24.7%)		
Crop Ceca	75 75	11 (14.7%) 1 (1.3%)	` /	12 (16%) 1 (1.3%)		

Data are shown as a percentage of positive samples

of Arcobacter in the drinking water (Table 2). For the farm receiving chlorinated city water, Arcobacter spp. were not recovered from drinker samples. However, A. butzleri was readily isolated from all water samples from farms 3 and 4, which received non-chlorinated well water. For the single premise (Farm 5) for which chlorination was not done that day, 50% of drinker water samples yielded Arcobacter. A. butzleri was recovered from every Arcobacter-positive water sample and Arcobacter spp. other than A. butzleri, only twice from contaminated drinkers. During resampling of these same premises in early spring (Table 3), the absence of A. butzleri in the drinkers contrasts with its recovery from 15% of crops (n = 75) and from cecal contents of a single turkey (1.3%, n = 75). This could be the result of inadequate sample volume or sample numbers, low levels of A. butzleri in water, sampling protocols, or that contaminated water is not a vehicle of Arcobacter transmission in turkeys. Changes in ambient temperature may contribute to the observed seasonal variations in A. butzleri contamination of the drinkers. For our study



b ND = Not determined

¹ Isolates were confirmed as *Arcobacter* spp. but did not amplify with the *A. butzleri*-specific primers in the multiplex PCR assay. These are most probably *A. cryaerophilus*

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farms, ambient daytime summer high temperatures range from 82 to 86°F with relative humidity above 80%. Average daytime highs during the spring fluctuate between 48 and 72°F with a 77% relative humidity (www.nws.noaa.gov). Whether these reflect differential thermotolerance of A. butzleri and A. cryaerophilus is not known. Nevertheless, the hypothesis that non-chlorinated water may be a reservoir of Arcobacter on turkey farms as has been suggested previously for human transmission (Kiehlbauch et al. 1991a; Lerner et al. 1994; Rice et al. 1999) awaits more rigorous sampling on a larger scale than that attempted in this study. Interestingly, Campylobacter spp. prevalence, based on cloacal swabs of these same birds, ranged from 65 to 80% despite the chlorination status of water (Wesley et al. 2005).

Although our multiplex PCR assay did not differentiate A. cryaerophilus (Harmon and Wesley 1997) its frequency in poultry-associated samples such as transport crates and carcasses suggests that the non-butzleri species identified in our studies was A. cryaerophilus. To illustrate, Son et al. (2006) earlier reported Campylobacter overall on 79% of broiler carcasses sampled at pre-scald as well as at pre- and post-chill. Arcobacter was isolated from 55% of these samples with butzleri (79.1%) the predominant species followed by A. cryaerophilus (20.9%).

In the current study, A. butzleri prevalence estimates for cecal contents for summer 2003

(2.86%, 2 of 70 samples, Table 1) and spring 2004 (1.33%, 1 of 75 samples, Table 3) were comparable. Thus, Arcobacter infrequently or transiently colonizes the ceca of commercial turkeys, as Eifert (2003) observed for experimentally inoculated chickens (n = 360) and may not be part of the normal avian intestinal flora as suggested by others (Atabay et al. 2003). Yet in two unrelated studies, Arcobacter was reported in 15% of cloacal swabs of chickens and older layer hens (Kabeya et al. 2003; Wesley and Baetz 1999). Type of birds (chicken layer hens, turkeys), age sampled, levels of detectable Arcobacter excreted into the cloaca and sensitivity of the different isolation or PCR-based protocols may impact prevalence estimates.

As summarized in Table 4, during summer 2004 we did not detect *Arcobacter* in environmental samples collected on Farm 1, including walls, cooling fans, and litter. In contrast, *A. butz-leri* was identified in 96% of carcass swabs (48 of 50 samples) originating from this flock. Farm 2, similarly failed to yield *Arcobacter* in either water or litter whereas it was present in 88% of carcass swabs. An outbreak of pneumovirus on Farm 3 precluded on-farm environmental sampling. Yet at slaughter, 94% of carcasses from this flock yielded *Arcobacter* spp., including *A. butzleri* (22%) and non-*butlzeri* (72%). These data agree with our earlier sampling in which 51% of whole turkey carcass rinses (n = 203) yielded

Table 4 Summary of distribution of Arcobacter in the farm environment and on carcasses: Summer 2004

Sample					
Туре	Number	Number Positive (% positive)		Total	
		A. butzleri ¹	Arcobacter spp. ¹		
Farm 1					
Environmental	25	0	0	0	
Carcass	50	48 (96%)	0	48 (96%)	
Farm 2		· ,		` ′	
Environmental	25	0	0	0	
Carcass	50	42 (84%)	2 (4%)	44 (88%)	
Farm 3		` ,	` ,	` /	
Environmental	Not Determined				
Carcass	50	11 (22%)	36 (72%)	47 (94%)	

Data are shown as a percentage of positive samples

¹ Isolates, which were confirmed as *Arcobacter* spp. but did not amplify with the *A. butzleri*-specific primers in the multiplex PCR assay. These are most probably *A. cryaerophilus*



Campylobacter; Arcobacter spp., primarily A. butzleri (65%), was isolated from 79% of carcasses (n = 309). In this study, the paucity of A. butzleri in cloacal swabs (2.0%, n = 298), cecal contents (2.9%, n = 145), and feathers (2.7%, n = 75) contrasts with its overall recovery from 93% of carcasses of market weight turkeys (n = 150). Low levels of Arcobacter on-farm but its frequent detection on turkey carcasses support the earlier hypothesis that the plant is a reservoir for poultry carcass contamination (Gude et al. 2005; Houf et al. 2003).

Conclusion

In this first description in market weight turkeys, *Arcobacter* was infrequently detected in the intestine, including cloacal swabs (2%, 6 of 298 samples) and cecal contents (2.1%, 3 of 145 samples). *Arcobacter* was readily identified from carcass swabs at slaughter (93%, 139 of 150 samples). The microbe was identified in drinkers in houses supplied with non-chlorinated water. This concurs with the observation for broilers that *Arcobacter* is uniquely adapted to survive in water, including that of the humid environment of the abattoir, where it may readily contaminate the carcass of the freshly slaughtered bird.

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